



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : A61K 31/44	A1	(11) International Publication Number: WO 91/02527 (43) International Publication Date: 7 March 1991 (07.03.91)
(21) International Application Number: PCT/US90/04637 (22) International Filing Date: 15 August 1990 (15.08.90) (30) Priority data: 396,523 21 August 1989 (21.08.89) US 494,744 16 March 1990 (16.03.90) US (71) Applicant: BETH ISRAEL HOSPITAL ASSOCIATION [US/US]; 330 Brookline Avenue, Boston, MA 02215 (US). (72) Inventors: SHARPE, Richard, J. ; 30 Melbourne Avenue, Newtonville, MA 02160 (US). ARNDT, Kenneth, A. ; 104 Lake Avenue, Newton Centre, MA 02159 (US). GALLI, Stephen, J. ; 9 Lakeview Terrace, Winchester, MA 01890 (US).		(74) Agent: CLARK, Paul, T.; Fish & Richardson, One Financial Center, Suite 2500, Boston, MA 02111-2658 (US). (81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent)*, DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), NO, SE (European patent). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: METHOD AND COMPOSITION FOR THE TREATMENT OF CUTANEOUS, OCULAR, AND MUCOSAL HYPERSENSITIVITY, INFLAMMATION, AND HYPERPROLIFERATIVE CONDITIONS USING TOPICAL PREPARATIONS OF SEROTONIN ANTAGONISTS (57) Abstract A method and composition for the topical treatment of cutaneous, mucosal or ocular hypersensitivity reactions, inflammation, or epithelial hyperproliferative states, including those associated with scarring. The composition, to be applied directly to an affected area of the skin, eye, or mucosal membrane, consists of a therapeutic amount of reserpine, spiperone, or other serotonin antagonist which has been incorporated into a vehicle suitable for topical administration.		

DESIGNATIONS OF "DE"

Until further notice, any designation of "DE" in any international application whose international filing date is prior to October 3, 1990, shall have effect in the territory of the Federal Republic of Germany with the exception of the territory of the former German Democratic Republic.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MC	Monaco
AU	Australia	FI	Finland	MG	Madagascar
BB	Barbados	FR	France	ML	Mali
BE	Belgium	GA	Gabon	MR	Mauritania
BF	Burkina Fasso	GB	United Kingdom	MW	Malawi
BG	Bulgaria	GR	Greece	NL	Netherlands
BJ	Benin	HU	Hungary	NO	Norway
BR	Brazil	IT	Italy	PL	Poland
CA	Canada	JP	Japan	RO	Romania
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	LI	Liechtenstein	SN	Senegal
CM	Cameroon	LK	Sri Lanka	SU	Soviet Union
DE	Germany	LU	Luxembourg	TD	Chad
DK	Denmark			TC	Togo
				US	United States of America

-1-

METHOD AND COMPOSITION FOR THE TREATMENT OF CUTANEOUS,
OCULAR, AND MUCOSAL HYPERSENSITIVITY, INFLAMMATION,
AND HYPERPROLIFERATIVE CONDITIONS USING TOPICAL
PREPARATIONS OF SEROTONIN ANTAGONISTS

Background of the Invention

This application is a continuation-in-part of
Sharpe et al. U.S.S.N. 07/396,523, filed August 21,
1989, hereby incorporated by reference.

5 The field of the invention is topical treatment
of cutaneous, ocular, and mucosal hypersensitivity,
inflammation, and hyperproliferative conditions.

 Cutaneous, ocular, and mucosal inflammation,
the development of changes in vascular tone and
10 permeability and the associated infiltration of the
skin, ocular, or mucosal tissues by leukocytes in
response to endogenous or exogenous stimuli, probably
evolved as a defense mechanism against infectious
agents. However, even in healthy adults, cutaneous,
15 ocular, and mucosal inflammation can occur in response
to certain plant resins, such as those of poison ivy,
and other commonly encountered agents in the
environment. In individuals sensitized to these agents,
a severe contact reaction can result upon exposure, with
20 significant associated morbidity. Inflammation also
occurs in association with reactions to physical agents
such as sunlight and in association with thermal,
electrical, or chemical burns. Severe or repeated
inflammatory reactions can be followed by significant
25 chronic changes, such as scarring of affected tissues.
In some anatomical sites, such as the eye, these chronic
changes can have serious long-term consequences,
including diminished vision or actual blindness.

-2-

It is now widely recognized that much cutaneous, ocular, and mucosal inflammation is pathological in nature. For example, in atopic dermatitis and eczema in general, leukocyte
5 (particularly mononuclear cells, lymphocytes, neutrophils, and eosinophils) infiltration into the skin is a general phenomenon and is important in the pathogenesis of these diseases. Similarly, psoriasis, a common cutaneous disease associated with a
10 hyperproliferating epidermis, also has an inflammatory component. It is now believed that cells found in the normal and abnormal skin, eye, or mucosal membrane secrete cytokines which are important in recruiting inflammatory cells into these sites and in inducing
15 chronic changes such as scarring.

In addition to contact dermatitis, atopic dermatitis, and eczema, other conditions involving pathogenic cutaneous, ocular, and mucosal inflammation include, but are not limited to psoriasis, ichthyosis,
20 acne vulgaris, alopecia areata, male and female pattern alopecia, arthropod bite reactions, pyoderma gangrenosa, lichen planus, cutaneous lupus erythematosus, scleroderma, mycosis fungoides, drug eruptions, and burns. These conditions may result in any one or more
25 of the following symptoms or signs: itching, swelling, reddening, blisters, crusting, pain, scaling, cracking, hair loss, scarring, or oozing of fluid involving the skin, eye, or mucosal membranes.

The potential therapeutic benefits of
30 controlling pathological cutaneous, ocular, or mucosal hypersensitivity, inflammation, hyperproliferation, or scarring has led to a search for therapeutic agents which are both safe and effective. Several substances are known to have the capability of inhibiting cutaneous
35 leukocyte responses or hyperproliferative responses.

-3-

Corticosteroids when administered systemically are effective in this regard, but are associated with significant and potentially dangerous side effects. Topically applied corticosteroids have some efficacy in treating these conditions, but are only partially effective in many instances, and have their own significant side effects. Cyclosporine A when given systemically is also partially efficacious, but of little or no utility when applied topically. Cyclosporine A is also associated with the real potential of serious toxicity to several major organ systems. Other agents with partial utility for treating some of the above conditions include, psoralen plus ultraviolet A (PUVA), dapsone, and anti-malarials, but the risk-to-benefit ratios for these agents is unfavorable for most of these conditions.

There is a significant and very long-standing need to identify agents which can be applied topically to prevent or suppress (i.e. "treat") cutaneous, ocular, or mucosal hypersensitivity reactions, inflammation, hyperproliferation, or scarring, and which have favorable benefit to risk ratios. Optimally such agents should primarily act locally, and systemic absorption should not result in blood levels high enough to cause significant systemic toxicity.

It is an object of the present invention to present a method for the topical treatment of reactions of cutaneous, mucosal or ocular hypersensitivity.

It is another object of the present invention to present a method for the topical treatment of cutaneous, mucosal, or ocular inflammation.

It is yet another object of the present invention to present a method for the topical treatment of cutaneous, mucosal, or ocular epithelial hyperproliferation.

-4-

It is yet another object of the invention to present a method for the topical treatment of cutaneous, mucosal or ocular scarring.

5 It is further an object of the present invention to present a composition for the topical treatment of cutaneous, ocular, or mucosal hypersensitivity reactions, inflammation, hyperproliferation, or scarring, which composition contains a therapeutic amount of a rauwolfia alkaloid
10 (an alkaloid derived from the Rauwolfia genus of plants) such as reserpine (a serotonin antagonist) or related compounds such as other serotonin antagonists which include but are not limited to ketanserin, cyproheptadine, spiperone, methysergide, LY 53857 (Lum
15 and Piercey, Eur. J. Pharmacol. 149:9-15, 1988), ritanserin, ICI 169-369 (Goldstein et al., J. Pharmacol. Exp. Ther. 249:673-680, 1989), risperidone, pipamperone, trazodone, cinanserine, mianserin, and LY 281067 (Foreman et al., Life Sciences 45:1263-1270, 1989).

20 It is more specifically an object of the present invention to present a composition for the topical treatment of cutaneous, ocular or mucosal hypersensitivity reactions, inflammation, hyperproliferation, or scarring, which composition
25 consists of a therapeutic amount of a rauwolfia alkaloid, or a serotonin antagonist selected from the group consisting of reserpine, ketanserin, cyproheptadine, spiperone, methysergide, LY 53857, ritanserin, ICI 169-369, risperidone, pipamperone,
30 trazodone, cinanserine, mianserin, LY 281067, and analogues and derivatives thereof, dissolved or suspended in an preparation appropriate for topical administration.

-5-

It is further an object of the present invention to present a method and composition for the topical treatment of cutaneous, ocular, or mucosal hypersensitivity reactions, inflammation,
5 hyperproliferation or scarring, in a fashion that limits significant systemic effects.

Brief Summary of the Invention

The subject invention concerns novel topical compositions and methods for the inhibition of
10 cutaneous, ocular or mucosal hypersensitivity reactions, inflammation, hyperproliferation, or scarring. The preferred composition described herein comprises a rauwolfia alkaloid or serotonin antagonist, such as reserpine, in vehicles suitable for topical application
15 and cutaneous, ocular or mucosal absorption. In tests conducted in accordance with the present invention, many such compositions have been shown to be effective in inhibiting cutaneous contact hypersensitivity reactions at the site of topical application, at doses that
20 produce little or no inhibition of this response at a site distant from the site of topical application. In addition, topical application of a composition including spiperone induces inhibition of cutaneous contact hypersensitivity or inflammatory reactions at both the
25 site of application and at a distant site, indicating that this particular serotonin antagonist may have significant systemic activity when applied to a cutaneous surface.

In accordance with the present invention, it
30 has been discovered that the properties of reserpine, spiperone, and other related compounds, such as other serotonin antagonists, make them useful as topical agents in treating contact dermatitis, atopic

-6-

dermatitis, eczematous dermatitis, drug eruptions, lichen planus, ichthyosis, pyoderma gangrenosa, psoriasis, alopecia areata, male and female pattern alopecia, cutaneous lupus erythematosus, scleroderma, inflammatory acne, arthropod bite reactions, aphthous ulcer, conjunctivitis, iritis, keratoconjunctivitis, vaginitis, proctitis, chemical burns, thermal burns, and photosensitivity conditions including sunburn. The novel method may also be useful in reducing the infiltration of skin by malignant leukocytes in diseases such as mycosis fungoides.

In its broadest overall aspect the composition is simply a rauwolfia alkaloid or a serotonin antagonist, of the type described above, dissolved or suspended in a suitable carrier. The method is to apply the composition directly onto the affected area of the skin, eye, or mucosal membrane.

Brief Description of the Drawings

Fig. 1 is a graph which shows the effects of topical reserpine on cutaneous contact hypersensitivity reactions (inflammation). The x axis represents time points 0, 24 and 48 hours after challenging both ears of mice with oxazolone, and the y axis is measurement of total ear thickness.

Fig. 2 is a bar graph illustrating the effect of topical administration of spiperone on ear swelling, expressed as changes (Δ) in ear thickness (post challenge value minus baseline prechallenge value) in $\text{mm} \times 10^{-2}$, associated with oxazolone-induced contact hypersensitivity.

Fig. 3 is a bar graph illustrating the effect of topical administration of spiperone on numbers of infiltrating inflammatory cells, expressed as number of inflammatory cells per mm^2 of dermis, associated with oxazolone-induced contact hypersensitivity.

Fig. 4 is a bar graph illustrating the effect of topical administration of spiperone on ear swelling (expressed as in Fig. 2) associated with DNFB-induced contact hypersensitivity.

Fig. 5 is a bar graph illustrating the effect of topical administration of spiperone on numbers of infiltrating inflammatory cells (expressed as in Fig. 3) associated with DNFB-induced contact hypersensitivity.

Fig. 6 is a bar graph illustrating the effect of topical administration of spiperone on numbers of infiltrating inflammatory cells (expressed as in Fig. 3) associated with IL-1-induced inflammation.

Detailed Description of the Invention

The subject invention is based on the discovery that cutaneous, ocular, or mucosal hypersensitivity reactions, inflammation, epithelial hyperproliferation, or scarring, can be treated by topical formulations of reserpine and/or spiperone (serotonin antagonists), and related compounds. Moreover, for many of these compounds, this effect can be directed to the site of application and immediate surrounding area without a significant similar systemic effect.

The conditions that the subject invention is therapeutically beneficial in treating include cutaneous hypersensitivity/inflammatory conditions such as contact dermatitis, atopic dermatitis, eczematous dermatitis, lichen planus, drug eruptions, cutaneous lupus erythematosus, scleroderma, pyoderma gangrenosa, alopecia areata, male and female pattern alopecia,

-8-

inflammatory acne, arthropod bite reactions, burns, and photosensitivity conditions, including sunburn; cutaneous epidermal hyperproliferative conditions such as psoriasis and ichthyosis; and mucosal hypersensitivity/ inflammatory conditions such as lichen planus, aphthous ulcers, vaginitis, proctitis, conjunctivitis, iritis and keratoconjunctivitis. Additionally, suppression of a chronic inflammatory condition can prevent or lessen scar formation caused by the inflammation.

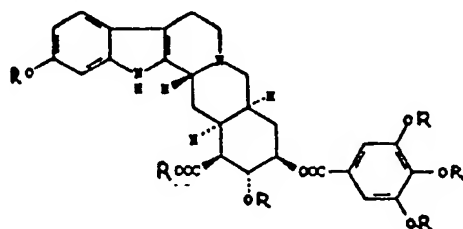
10 The subject invention pertains not only to reserpine, a rauwolfia alkaloid with serotonin antagonist properties, and spiperone, another serotonin antagonist, but also to other related compounds which have similar biologic activity with respect to the control of inflammation, function, migration, and proliferation of cutaneous and mucosal cells. Other such compounds may include, but are not limited to other rauwolfia alkaloids and serotonin antagonists such as ketanserin, cyproheptadine, methysergide, LY 53857, ritanserin, ICI 169-369, risperidone, pipamperone, 20 trazodone, cinanserin, mianserin, LY 281067, and analogues and derivatives thereof.

 Reserpine and some of the above related compounds are presently used for treating hypertension and psychiatric disorders. Reserpine, which depletes 25 stores of serotonin (5-hydroxy-tryptamine) in many organs, has been shown to be effective in suppressing hypersensitivity responses in rodents when administered in high doses systemically. The physical properties of reserpine, spiperone, and the other related compounds 30 are well documented. By applying these agents topically, therapeutic local concentrations are attainable without, for many of the compounds, the associated systemic side effects. Spiperone, however,

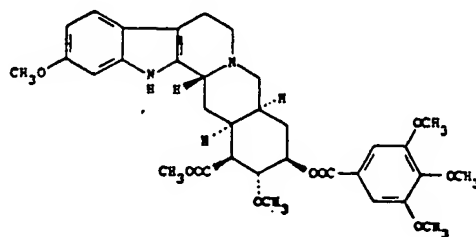
-9-

under certain conditions (e.g., high doses) has been found to act to suppress hypersensitivity and inflammation both systematically and locally, when applied as a topical preparation to cutaneous surfaces.

5 Reserpine that is used in the present invention can be generally represented by the formula:



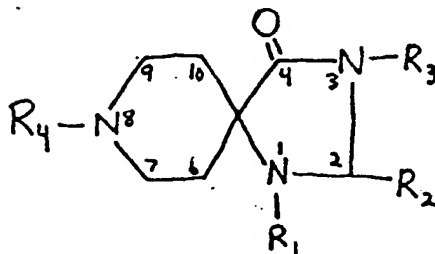
wherein each R = a hydrogen or alkyl of 1 to 6 carbon atoms. The form of reserpine utilized in Example 1 has the formula:



10 The term "spiperone" herein denotes all of the molecules which are effective in the method of the invention and which are the subject of the following U.S. patents: No. 3,155,669; No. 3,155,670; No. 3,161,644; and No. 3,238,216; all of which patents are
15 hereby incorporated by reference. Methods for the synthesis of each such compound are disclosed in said four patents.

-10-

More particularly, forms of spiperone having the following formulae may be employed in the method of the invention:



wherein

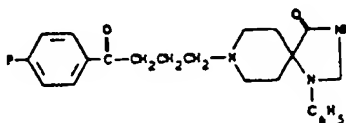
- 5 $R_1 = \text{H}, \text{CH}_3-, \text{C}_6\text{H}_5-, \text{cyclohexyl}, 4-(\text{OCH}_3)\text{C}_6\text{H}_4-,$
 $3-(\text{CH}_3)\text{C}_6\text{H}_4-, 4-(\text{CH}_3)\text{C}_6\text{H}_4-, 4-\text{X}-\text{C}_6\text{H}_4-,$
 $(\text{CH}_3)_2\text{CH}-, \text{CH}_3(\text{CH}_2)_3-, (\text{CH}_3)_2\text{CHCH}_2-,$
 $\text{CH}_3\text{CH}_2\text{CH}(\text{CH}_3)-, \text{or } (\text{CH}_3)_3\text{C}-;$
- $R_2 = \text{H or CH}_3;$
- 10 $R_3 = \text{H}, \text{CH}_3, \text{CH}_3\text{CH}_2-, \text{CH}_3\text{CH}_2\text{CH}_2-, (\text{CH}_3)_2\text{CH}-, \text{or}$
 $\text{CN}(\text{CH}_2)_2-;$
- $R_4 = \text{H}, \text{C}_6\text{H}_5\text{CH}(\text{CH}_2\text{CH}_3)\text{CH}_2-, \text{C}_6\text{H}_5\text{CH}(\text{CH}_3)(\text{CH}_2)_2-,$
 $\text{C}_6\text{H}_5\text{CH}_2\text{CH}(\text{CH}_3)\text{CH}_2-, \text{C}_6\text{H}_5\text{CH}_2\text{CH}_2\text{CH}(\text{CH}_3)-,$
 $\text{C}_6\text{H}_5\text{CH}(\text{CH}_3)(\text{CH}_2)_3-, 4-\text{CH}_3\text{C}_6\text{H}_4\text{CH}(\text{CH}_3)(\text{CH}_2)_3-,$
15 $4-(\text{CH}_3\text{O})\text{C}_6\text{H}_4\text{CH}(\text{CH}_3)(\text{CH}_2)_3, 4-\text{X}-\text{C}_6\text{H}_4\text{CH}(\text{CH}_3)\text{CH}_2-,$
 $4-\text{X}-\text{C}_6\text{H}_4\text{CH}(\text{CH}_2\text{CH}_3)\text{CH}_2-, 4-\text{X}-\text{C}_6\text{H}_4\text{CH}(\text{CH}_3)(\text{CH}_2)_2-,$
 $4-\text{X}-\text{C}_6\text{H}_4-\text{CH}(\text{CH}_3)(\text{CH}_2)_3-, \text{C}_6\text{H}_5\text{CH}(\text{OCH}_3)(\text{CH}_2)_2-,$
 $\text{C}_6\text{H}_5\text{CH}-\underset{\text{CH}_2}{\text{CH}}-\text{CH}_2-, \text{C}_6\text{H}_5\text{CO}(\text{CH}_2)_3-, \text{C}_6\text{H}_5\text{CO}(\text{CH}_2)_4-,$
 $4-(\text{CH}_3)\text{C}_6\text{H}_4\text{CO}(\text{CH}_2)_3-, 4-(\text{CH}_3\text{O})\text{C}_6\text{H}_4\text{CO}(\text{CH}_2)_3-,$
20 $4-\text{X}-\text{C}_6\text{H}_4\text{CO}(\text{CH}_2)_3-, 4-\text{X}-\text{C}_6\text{H}_4\text{CO}(\text{CH}_2)_3-,$
 $2\text{-thienyl-CO}(\text{CH}_2)_3-, \text{or } \text{Ar}_1-\underset{\text{Ar}}{\text{CH}}(\text{CH}_2)_n-,$

wherein $n = 3 \text{ or } 4$; $\text{X} = \text{F}, \text{Cl}, \text{or Br}$; and each of Ar and Ar_1 is, independently, $\text{H}, \text{C}_6\text{H}_5-, 4-(\text{CH}_3)\text{C}_6\text{H}_4-,$
 $4-(\text{CH}_3\text{O})\text{C}_6\text{H}_4-, 4-\text{X}-\text{C}_6\text{H}_4-, 3-(\text{CX}_3)\text{C}_6\text{H}_4-, 2\text{-thienyl},$
25 $\text{or } 4-\text{X}-\text{C}_6\text{H}_4\text{CH}_2-.$

-11-

Those forms of spiperone which are particularly useful in the method of the invention include those in which R_1 is C_6H_5- , $4-(X)-C_6H_4-$ or $4-(CH_3)C_6H_4-$; R_2 is H or CH_3 ; R_3 is H, CH_3 , or CH_3CH_2- ; and R_4 is $4-X-C_6H_4CO(CH_2)_3-$ or 2-thienyl- $CO(CH_2)_3-$; and especially those in which R_1 is C_6H_5- , $4-(Br)-C_6H_4-$, or $4-(Cl)-C_6H_4-$; R_2 is H or CH_3 ; R_3 is H, CH_3 , or CH_3CH_2- ; and R_4 is $4-X-C_6H_4CO(CH_2)_3-$ or 2-thienyl- $CO(CH_2)_3-$.

The particular serotonin antagonist used in Examples 2-4 is 8-[3-(*p*-fluorobenzoyl)propyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one. Herein termed "spiperone (1)", this compound has the following structure:



The potential utility of any one of the above-described forms of spiperone as a topical immunosuppressant and/or anti-inflammatory agent can be conveniently determined by synthesizing the compound and testing it in one or more of the biological assays described in Examples 2-4.

Furthermore, the reserpine and spiperone of the subject invention, and the other related compounds showing similar biologic activity, can be modified in order to enhance their usefulness as pharmaceutical compositions. For example, it is well known in the art

-12-

that various modifications, such as alteration of charge, can alter water and lipid solubility and thus alter the potential for percutaneous absorption and for crossing the blood-brain barrier. The vehicle can be similarly modified to enhance cutaneous absorption, enhance the reservoir effect, and minimize potential irritancy or neuropharmacological effects of the composition. Additionally, the topical formulation can be occluded to further enhance absorption.

Preservatives, stabilizers, emulsifiers, emulsion stabilizers, antioxidants, chelating agents, solvents, thickening agents, emollients, and humectants may be necessary or useful as part of the topical formulation (Arndt, K.A., P.V. Mendenhall [1987]) The Pharmacology of Topical Therapy. In Dermatology in General Medicine. T.B. Fitzpatrick, A.Z. Eisen, K. Wolff, I.M. Freedberg and K.F. Austen, eds., 3d ed., McGraw Hill, Inc., New York, pp.2532- 2540). In addition, natural or artificial flavorings or sweeteners may be added to enhance the taste of topical preparations applied for local effect to mucosal surfaces. Inert dyes or colors may be added, particularly in the case of preparations designed for application to oral mucosal surfaces.

Suitable vehicles or carriers for topical application may contain a daily dose of between 0.1 milligrams and 120 grams of 0.001% to 100% (all percentages are by weight) of the active compound, and may be prepared by conventional techniques to be in conventional forms such as lotions, suspensions, ointments, creams, gels, tinctures, suppositories, elixirs, solutions, aerosols, sprays, powders, pastes or slow-release polymers for topical application; or mouth rinses or rectal or vaginal suppositories for local/topical application to these respective mucosal

-13-

surfaces. Suitable pharmaceutical diluents or carriers include water, alcohols, sterols, propylene glycol, polyethylene glycol, glycerin, diisopropyl adipate, 1,2,6-hexanetriol, isopropyl myristate, propylene carbonate, natural and/or synthetic or hardened oils and waxes, kaolin, talc, titanium dioxide, as well as suitable solubilizers or emulsifying agents such as ethyl-hydroxybenzoate, cholesterol, sodium laureth sulfate, sodium lauryl sulfate, sorbitan esters, cetyl alcohol, cetearyl alcohol, stearyl alcohol, or stearic acid. Stabilizers such as benzyl alcohol, sodium bisulfite, edetate disodium, citric acid, chlorocresol, butylated hydroxyanisole and butylated hydroxytoluene may be added. Thickening agents such as petrolatum, beeswax, xanthan gum, or polyethylene, plus humectants such as sorbitol solution may also be added. Similarly, emollients such as mineral oil, lanolin and its derivatives, or squalane can be included as part of the topical formulation. Natural or artificial sweeteners including glucose, fructose, sucrose, aspartame, or saccharin may be added to enhance the palatability of preparations applied to mucosal surfaces. Similarly, flavorings such as peppermint oil may be added. Inert dyes such as yellow dye number 6 may be added, particularly in the case of preparations designed for topical application to oral mucosal surfaces.

Example 1: Suppression of oxazolone-induced contact hypersensitivity by topically-applied reserpine.

One important composition of the subject invention contains about 3.7% by weight of reserpine (reserpine tablets, distributed by Schein Pharmaceuticals, Inc., Port Washington, NY, 11050 and

-14-

manufactured by Richlyn Labs of Philadelphia, PA, 19124). Reserpine was extracted from the tablets by dissolving the tablets in ethanol, centrifuging the suspension twice and lyophilizing the supernatant. The resultant dried powder was dissolved in about 47.5% ethanol, 10% water, 1% sodium laureth sulfate, 4% isopropyl alcohol, and 37.5% propylene glycol, to make a solution having about 3.7% reserpine by weight.

Fig. 1 demonstrates the therapeutic effect of 20µl of the above reserpine preparation on the expression of contact sensitivity reactions in the right ears of mice. Mice (C57BL/6J female mice, 6-8 weeks old: Jackson Laboratory, Bar Harbor, ME; or Balb/c female mice, 6-8 weeks old: Charles River Laboratories, Kingston Facility, Stoneridge, NY) were sensitized to 3% oxazolone (4-ethoxymethylene- 2-phenyl-oxazol-5-one) in 4:1 acetone/olive oil, by applying 50µl to the shaved abdomen and 5µl to each hind footpad of each mouse, seven days earlier. On the day of treatment both the inner and outer surfaces of both ears of each mouse were challenged with 10µl of 0.5% oxazolone in 4:1 acetone/olive oil (i.e. 20µl per ear). One hour after challenging, the control preparation or reserpine preparation was administered by applying 10µl of a given preparation to each side of a treated ear (i.e. 20µl per ear). Ear thicknesses of the mice were measured just before challenging with oxazolone (bars 10, 20, 30, and 40). Of those mice given the reserpine preparation only the right ears were treated (bars 80 and 120). The reserpine preparation reduced the oxazolone-induced inflammation (i.e. the contact sensitivity reaction to oxazolone) significantly in the right ears of treated mice at 24 hours (bar 80) and at 48 hours (bar 120) after challenge with oxazolone, as

-15-

compared to the right ears of mice treated with 20 μ l of the vehicle preparation without reserpine (bars 60 and 100). Similarly, the left ears of the mice treated on the right ear with reserpine showed no decrease in swelling (bars 70 and 110); ear thickness measurements of these ears were the same as those of ears treated with the vehicle preparation without reserpine (bars 60 and 100), or those of the corresponding left ears of the mice treated on the right ears with vehicle lacking reserpine (bars 50 and 90). The occurrence of undiminished swelling (increase in ear thickness) in the left ears of those mice treated with reserpine topically on the right ear shows that the effect of topical reserpine is a local, rather than a systemic, effect. Additionally, another vehicle preparation (not containing the active ingredient, reserpine) has been shown to be ineffective in suppressing the ear swelling response in either the treated or the untreated ear. This vehicle was composed of about 1.86% water, 1.06% glycerin, 0.026% peppermint oil, 0.026% saccharin, 17% polyethylene glycol 400 (PEG 400), 40% ethanol, 40% propylene glycol. The lack of suppression of ear swelling by two complex vehicles (lacking the active ingredient reserpine) is evidence that the serotonin antagonist reserpine is needed for this effect.

Example 2: Suppression of oxazolone-induced contact hypersensitivity by topically-applied spiperone.

A composition of 4% w/w spiperone (1) (Sigma Chemical Co., St. Louis, Mo.) in a vehicle for topical administration (prepared as a suspension of 4 ml absolute ethanol, 1 ml of propylene glycol, and 5 ml of olive oil) was utilized to test the ability of spiperone to suppress the contact hypersensitivity response when applied topically.

-16-

The abdomens of C57BL/6J mice were shaved with electric clippers. 50 μ l of 4% w/w of oxazolone (Sigma Chemical Co.) in a 4:1 v:v acetone:olive oil solution was applied to the shaved abdomen and 5 μ l was applied to each hind footpad of each mouse. Five to seven days later, mice were challenged for contact hypersensitivity to oxazolone by applying 10 μ l of the 0.4% oxazolone solution to both the inner and the outer surfaces of each of both ears of each mouse. At one hour after the oxazolone challenge, the right ear of each mouse was topically treated either with 4% spiperone in vehicle or with vehicle alone, by applying 10 μ l of the test solution to each surface of the ear (20 μ l total per ear). Before and at 24 hours after the oxazolone challenge, ear thicknesses were measured with a spring-loaded engineer's micrometer. After measurement, mice were sacrificed and specimens of tissue from the right ear were fixed in 10% buffered formalin for at least 48 hours, and then prepared for microscopic assessment of infiltrating cells using standard techniques of morphometry performed on paraffin-embedded and hematoxylin- and eosin-stained sections. Using an ocular grid, specimens were examined in a coded fashion and all morphologically-identified inflammatory cells were quantified.

Topical treatment with spiperone reduces the extent of oxazolone-induced cutaneous contact hypersensitivity at the site of spiperone treatment, whether such hypersensitivity is expressed as ear swelling (" Δ ear thickness") (Fig. 2), or by the degree of infiltration of inflammatory cells (Fig. 3). The Δ ear thickness after topical treatment with 4% spiperone was approximately 90% lower than the Δ ear thickness seen with the untreated control mice (Fig. 2),

-17-

while the number of inflammatory cells per mm² of biopsied ear tissue decreased by approximately 76% compared to the untreated control mice (Fig. 3). Topical treatment of the right ear with spiperone also
5 reduced ear swelling and leukocyte infiltration in the left (untreated) oxazolone-challenged ear (reductions of 60% and 22%, respectively, compared to control mice not treated with spiperone on either ear), indicating that
10 spiperone, at that dose level, can exert a systemic effect when applied to a skin surface.

Example 3: Suppression of DNFB-induced contact hypersensitivity by topically-applied spiperone.

The procedure described in Example 2 was
15 repeated, substituting (a) 0.2% 2,4-dinitro-1-fluorobenzene (dinitrofluorobenzene, DNFB) in acetone (v/v) for both sensitization and elicitation of contact hypersensitivity for the oxazolone solution of
Example 2, and (b) a 0.5% (w/w) solution of spiperone
20 (1) in absolute ethanol for the 4% solution of Example 2. As shown in Fig. 4, treatment of the right ear with 0.5% spiperone reduced swelling in that ear by 46% and in the left (untreated) ear by 28%, compared to the ears of control mice which received no spiperone treatment.
25 Fig. 5 illustrates the reduction in numbers of infiltrating inflammatory cells in mice treated with 0.5% spiperone on the right ear. Such cell counts were reduced by 71% in the right (treated) ear and 18% in the left (untreated) ear, compared to the counts in the ears
30 of untreated control mice.

-18-

Example 4: Suppression of rIL-1 induced inflammation
 by topically-applied spiperone.

0.05 ml of phosphate-buffered saline ("PBS";
GIBCO, Grand Island, NY) containing 200 units of
5 recombinant human interleukin-1 ("rIL-1"; Genzyme
Corporation, Cambridge, MA; specific activity:
100,000 units/ μ g) was injected intradermally into both
ears of each of 12 Balb/c mice. At one hour after
injection of the rIL-1, the right ear of each mouse was
10 topically treated either with 4% spiperone in vehicle
(absolute ethanol), or with vehicle alone, as in
Example 2, by applying 10 μ l of the test solution to
each surface of the ear (20 μ l total per ear). At
eight hours after rIL-1 injection, ear thicknesses were
15 measured with a spring-loaded engineer's micrometer, the
mice were sacrificed, and ear tissue was processed for
microscopic assessment of infiltrating inflammatory
cells as described in Example 2.

As shown in Fig. 6, treatment with 4% spiperone
20 is capable of inhibiting rIL-1-induced inflammatory cell
infiltration by over 90% compared to the control mice
who received no spiperone, whether such inflammatory
cell counts were measured in the right ear, which
received the topical spiperone application, or in the
25 left ear, which was not directly treated with spiperone,
but rather reflects the systemic activity of spiperone
applied to the right ear (or possibly the spread of the
drug to the left ear during grooming by the mice).

30

Other Embodiments

Topical preparations of one or more of
reserpine, ketanserin, cyproheptadine, spiperone,
methysergide, LY 53857, ritanserin, ICI 169-369,
risperidone, pipamperone, trazodone, cinanserine,

-19-

mianserin, LY 281067, and analogues and derivatives thereof, may be used in combination with other active compounds in order to enhance the topical preparation's anti-proliferative, anti-inflammatory, anti-hypersensitivity, or anti-scarring properties, or to achieve additional therapeutic effects such as relief of pain or itching. For example, a topical reserpine preparation, or a preparation including one or more of the other serotonin antagonists, may be combined with one or more anti-fungal agents, anti-bacterial agents, or compounds capable of inhibiting cutaneous leukocyte accumulation, such as topical corticosteroids and calcium channel blockers including nifedipine, verapamil, diltiazam, isradipine, McN-6186 bepridil, niludipine, perhexiline, nicardipine, flunarizine, nilvadipine, nisoldipine, nitrendipine, felodipine, cinnarazine, and nimodipine. In order to treat acne, a traditional acne drug such as erythromycin, clindamycin, benzoyl peroxide, or a retinoid could be included as part of the preparation. Another example would be the addition of an antifungal drug such as ketoconazole, itraconazole, clotrimazole, oxiconazole, sulconazole, econazole, other imidazoles, naftifine, ciclopirox olamine, or nystatin, for the treatment of dermatophyte or candida infection. For treatment of conditions associated with hair loss, minoxidil or other agents that promote hair growth could be included as part of the preparation.

In practice, a therapeutic daily dose of a preparation of reserpine or other serotonin antagonist is applied directly to the inflamed area of the skin, eye, or mucosal membrane, and in a short period of time the inflammation is decreased.

-20-

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

What is claimed is:

-21-

1 1. A method for the treatment of a cutaneous
2 or mucosal disease involving hypersensitivity,
3 inflammation, scarring, or epithelial hyperproliferation
4 in an animal, said method comprising applying a
5 therapeutically-effective amount of a serotonin
6 antagonist to an affected area of the skin or mucosal
7 membrane of said animal.

8 2. The method of claim 1, wherein said disease
9 is selected from the group consisting of contact
10 dermatitis, atopic dermatitis, eczematous dermatitis,
11 drug eruptions, arthropod bite reactions, inflammatory
12 acne, alopecia areata, male and female pattern alopecia,
13 lichen planus, pyoderma gangrenosa, cutaneous lupus
14 erythematosus, scleroderma, mycosis fungoides,
15 psoriasis, ichthyosis, burns, aphthous ulcer, vaginitis,
16 and proctitis.

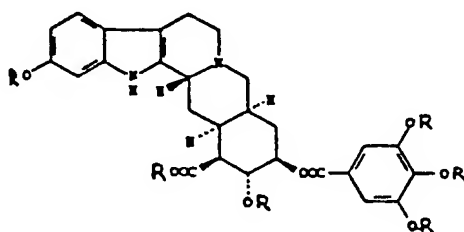
17 3. A method for the treatment of ocular
18 inflammation in an animal, said method comprising
19 applying a therapeutically-effective amount of a
20 serotonin antagonist to an affected area of the eye of
21 said animal.

22 4. A method for the prevention or reduction of
23 the formation of scar tissue in and around an eye of an
24 animal, said method comprising applying a
25 therapeutically-effective amount of a serotonin
26 antagonist to an affected area of said eye, or skin
27 surrounding said eye.

-22-

1 5. The method of claim 1, claim 3, or claim 4,
 2 wherein said serotonin antagonist is selected from the
 3 group consisting of reserpine, ketanserin,
 4 cyproheptadine, spiperone, methysergide, LY 53857,
 5 ritanserin, ICI 169-369, risperidone, pipamperone,
 6 trazodone, cinanserin, mianserin, and LY 281067.

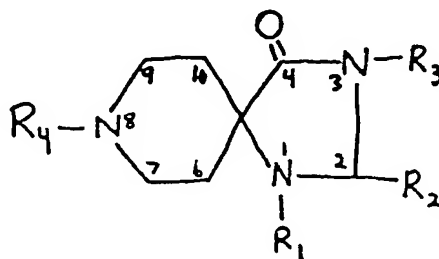
7 6. The method of claim 5 wherein said
 8 serotonin antagonist is reserpine, said reserpine having
 9 the chemical structure:



10 wherein each R = a hydrogen or an alkyl of 1 to 6 carbon
 11 atoms.

12 7. The method of claim 5, wherein said
 13 serotonin antagonist is spiperone.

14 8. The method of claim 7, wherein said
 15 spiperone has the chemical structure:



-23-

1 wherein

2 $R_1 = H, CH_3-, C_6H_5-, cyclohexyl, 4-(OCH_3)C_6H_4-,$
3 $3-(CH_3)C_6H_4-, 4-(CH_3)C_6H_4-, 4-X-C_6H_4-,$
4 $(CH_3)_2CH-, CH_3(CH_2)_3-, (CH_3)_2CHCH_2-,$
5 $CH_3CH_2CH(CH_3)-, or (CH_3)_3C-;$

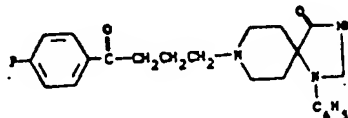
6 $R_2 = H \text{ or } CH_3;$

7 $R_3 = H, CH_3, CH_3CH_2-, CH_3CH_2CH_2-, (CH_3)_2CH-,$ or
8 $CN(CH_2)_2-$

9 $R_4 = H, C_6H_5CH(CH_2CH_3)CH_2-, C_6H_5CH(CH_3)(CH_2)_2-,$
10 $C_6H_5CH_2CH(CH_3)CH_2-, C_6H_5CH_2CH_2CH(CH_3)-,$
11 $C_6H_5CH(CH_3)(CH_2)_3-, 4-CH_3C_6H_4CH(CH_3)(CH_2)_3-,$
12 $4-(CH_3O)C_6H_4CH(CH_3)(CH_2)_3-, 4-X-C_6H_4CH(CH_3)CH_2-,$
13 $4-X-C_6H_4CH(CH_2CH_3)CH_2-, 4-X-C_6H_4CH(CH_3)(CH_2)_2-,$
14 $4-X-C_6H_4-CH(CH_3)(CH_2)_3-, C_6H_5CH(OCH_3)(CH_2)_2-,$
15 $C_6H_5CH-CH-CH_2-, C_6H_5CO(CH_2)_3-, C_6H_5CO(CH_2)_4-,$
16 $4-(CH_3)C_6H_4CO(CH_2)_3-, 4-(CH_3O)C_6H_4CO(CH_2)_3-,$
17 $4-X-C_6H_4CO(CH_2)_3-, 4-X-C_6H_4CO(CH_2)_3-,$
18 $2-thienyl-CO(CH_2)_3-, \text{ or } Ar_1-CH(CH_2)_n-,$
 Ar

19 wherein n = 3 or 4; X = F, Cl, or Br; and each of Ar and
20 Ar₁ is, independently, H, C₆H₅-, 4-(CH₃)C₆H₄-,
21 4-(CH₃O)C₆H₄-, 4-X-C₆H₄-, 3-(CX₃)C₆H₄-, 2-thienyl,
22 or 4-X-C₆H₄CH₂-.

23 9. The method of claim 8, wherein said spiperone
24 has the chemical structure:



-24-

1 10. The method of claim 1, claim 3, or
2 claim 4, wherein a daily dose of between 0.1 milligrams
3 and 120 grams of a topical preparation of said serotonin
4 antagonist containing between 0.001% and 100% of said
5 serotonin antagonist by weight is applied to said
6 affected area.

7 11. The method of claim 1, claim 3, or
8 claim 4, wherein said animal is a human.

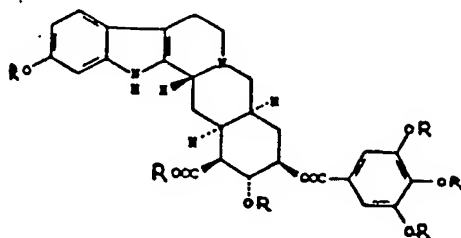
9 12. The method of claim 1, claim 3, or
10 claim 4, wherein said animal is a domestic animal kept
11 for companionship or commercial purposes.

12 13. A composition for the treatment of
13 cutaneous, ocular, or mucosal hypersensitivity
14 reactions, inflammation, or hyperproliferation, or the
15 prevention or reduction of associated scar tissue
16 formation in an animal, said composition comprising at
17 least 0.001% by weight of a serotonin antagonist
18 incorporated into a vehicle suitable for topical
19 application directly onto an affected area of said
20 animal.

21 14. The composition of claim 13, wherein said
22 serotonin antagonist is selected from a group consisting
23 of reserpine, ketanserin, cyproheptadine, spiperone,
24 methysergide, LY 53857, ritanserin, ICI 169-369,
25 risperidone, pipamperone, trazodone, cinanserine,
26 mianserin, LY 281067, and analogues and derivatives
27 thereof.

-25-

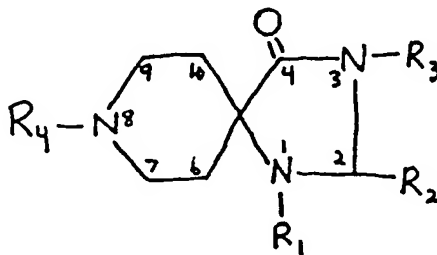
15. The composition of claim 14, wherein said serotonin antagonist is reserpine, said reserpine having the chemical structure:



wherein each
R = a hydrogen or an alkyl of 1 to 6 carbon atoms.

16. The composition of claim 14, wherein said serotonin antagonist is spiperone.

17. The composition of claim 16, wherein said spiperone has the chemical structure:



wherein

$R_1 = \text{H, CH}_3\text{-, C}_6\text{H}_5\text{-, cyclohexyl, 4-(OCH}_3\text{)C}_6\text{H}_4\text{-, 3-(CH}_3\text{)C}_6\text{H}_4\text{-, 4-(CH}_3\text{)C}_6\text{H}_4\text{-, 4-X-C}_6\text{H}_4\text{-, (CH}_3\text{)}_2\text{CH-, CH}_3\text{(CH}_2\text{)}_3\text{-, (CH}_3\text{)}_2\text{CHCH}_2\text{-, CH}_3\text{CH}_2\text{CH(CH}_3\text{)-, or (CH}_3\text{)}_3\text{C-;}$

$R_2 = \text{H or CH}_3\text{;}$

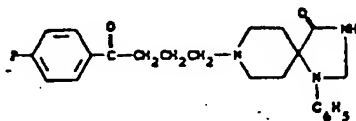
$R_3 = \text{H, CH}_3, \text{CH}_3\text{CH}_2\text{-, CH}_3\text{CH}_2\text{CH}_2\text{-, (CH}_3\text{)}_2\text{CH-, or CN(CH}_2\text{)}_2\text{-;}$

-26-

$$\begin{aligned}
1 \quad R_4 = & \text{H, } C_6H_5CH(CH_2CH_3)CH_2-, C_6H_5CH(CH_3)(CH_2)_2-, \\
2 \quad & C_6H_5CH_2CH(CH_3)CH_2-, C_6H_5CH_2CH_2CH(CH_3)-, \\
3 \quad & C_6H_5CH(CH_3)(CH_2)_3-, 4-CH_3C_6H_4CH(CH_3)(CH_2)_3-, \\
4 \quad & 4-(CH_3O)C_6H_4CH(CH_3)(CH_2)_3-, 4-X-C_6H_4CH(CH_3)CH_2-, \\
5 \quad & 4-X-C_6H_4CH(CH_2CH_3)CH_2-, 4-X-C_6H_4CH(CH_3)(CH_2)_2-, \\
6 \quad & 4-X-C_6H_4-CH(CH_3)(CH_2)_3-, C_6H_5CH(OCH_3)(CH_2)_2-, \\
7 \quad & C_6H_5\underset{\substack{| \\ CH_2}}{CH}-CH-CH_2-, C_6H_5CO(CH_2)_3-, C_6H_5CO(CH_2)_4-, \\
8 \quad & 4-(CH_3)C_6H_4CO(CH_2)_3-, 4-(CH_3O)C_6H_4CO(CH_2)_3-, \\
9 \quad & 4-X-C_6H_4CO(CH_2)_3-, 4-X-C_6H_4CO(CH_2)_3-, \\
10 \quad & 2\text{-thienyl-CO(CH}_2)_3-, \text{ or } Ar_1-\underset{\substack{| \\ Ar}}{CH}(CH_2)_n-,
\end{aligned}$$

11 wherein n = 3 or 4; X = F, Cl, or Br; and each of Ar and
12 Ar₁ is, independently, H, C₆H₅-, 4-(CH₃)C₆H₄-,
13 4-(CH₃O)C₆H₄-, 4-X-C₆H₄-, 3-(CX₃)C₆H₄-, 2-thienyl,
14 or 4-X-C₆H₄CH₂-.

15 18. The composition of claim 17, wherein said
16 spiperone has the chemical structure:



17 19. The composition of claim 13, wherein said
18 composition further comprises one or more additional
19 components capable of inhibiting cutaneous leukocyte
20 accumulation, said additional component(s) being
21 selected from a group consisting of corticosteroids;
22 calcium channel blockers including nifedipine,
23 verapamil, diltiazam, isradipine, McN-6186, bepridil,

-27-

1 niludipine, perhexiline, nicardipine, flunarizine,
2 nilvadipine, nisoldipine, nitrendipine, felodipine,
3 cinnarazine, and nimodipine; and other serotonin
4 antagonists such as ketanserin, cyproheptadine,
5 spiperone, methysergide, LY 53857, ritanserin, ICI
6 169-369, risperidone, pipamperone, trazodone,
7 cinanserine, mianserin, LY 281067, and analogues and
8 derivatives thereof.

9 20. The composition of claim 13, wherein said
10 composition further comprises an additional component
11 which is active against a fungal infection, said
12 additional component being selected from a group
13 consisting of ketoconazole, itraconazole, clotrimazole,
14 oxiconazole, sulconazole, econazole, other imidazoles,
15 naftifine, ciclopirox olamine, and nystatin.

16 21. The composition of claim 11, wherein said
17 composition further comprises an additional component
18 which promotes hair growth.

19 22. The composition of claim 19, wherein said
20 additional component is minoxidil.

1/3

FIG. 1

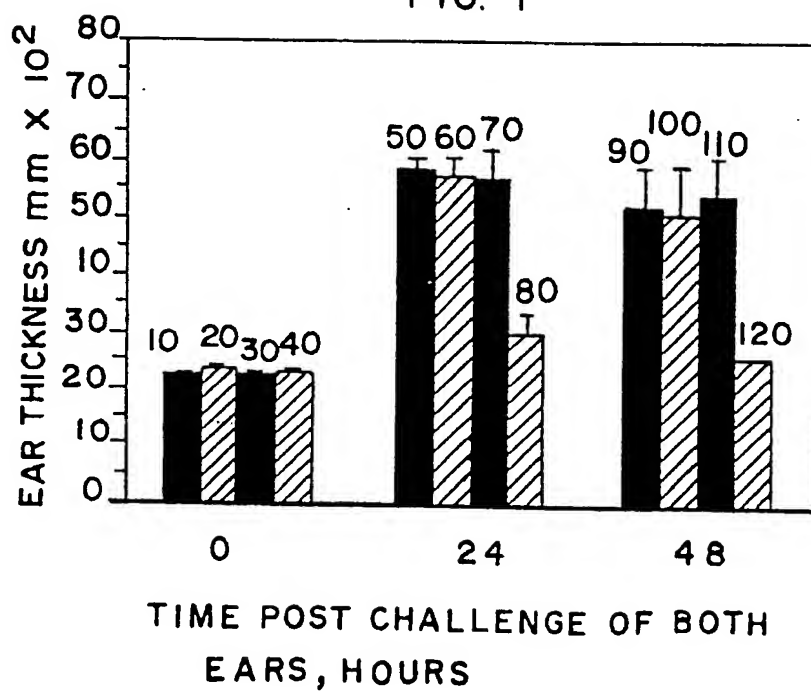
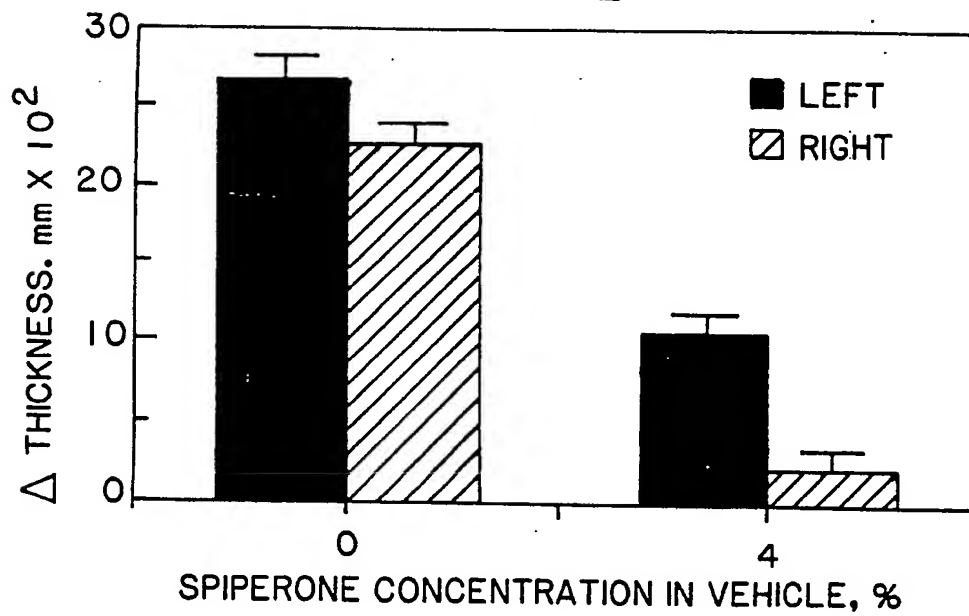


FIG. 2



SUBSTITUTE SHEET

2/3

FIG. 3

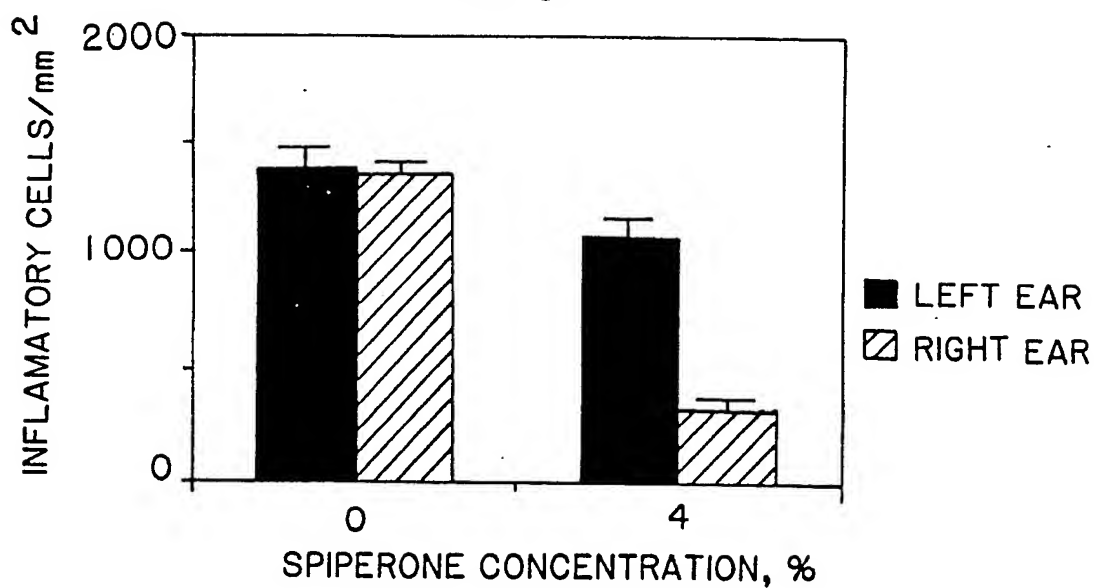
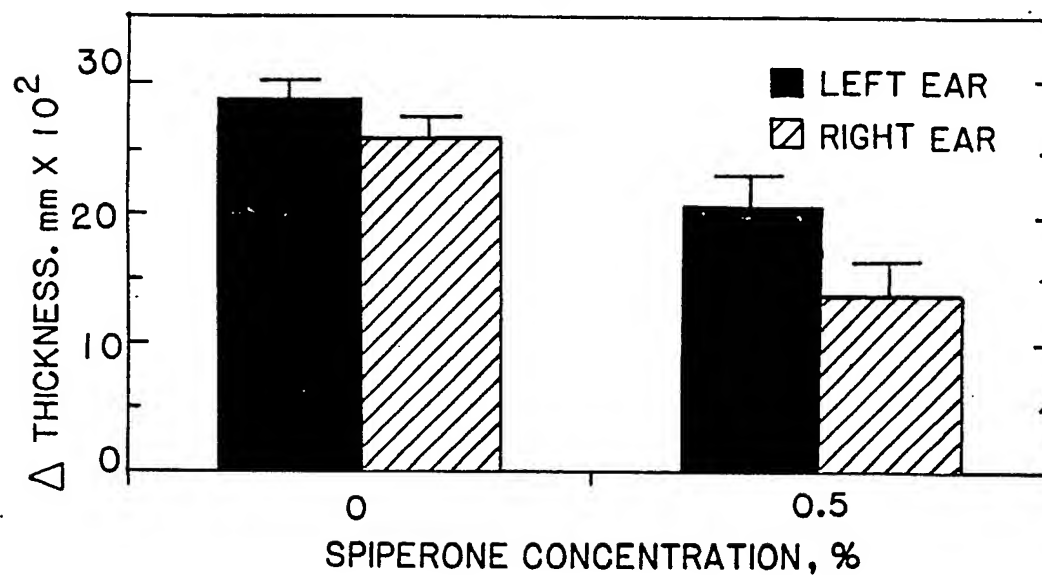


FIG. 4



3/3

FIG. 5

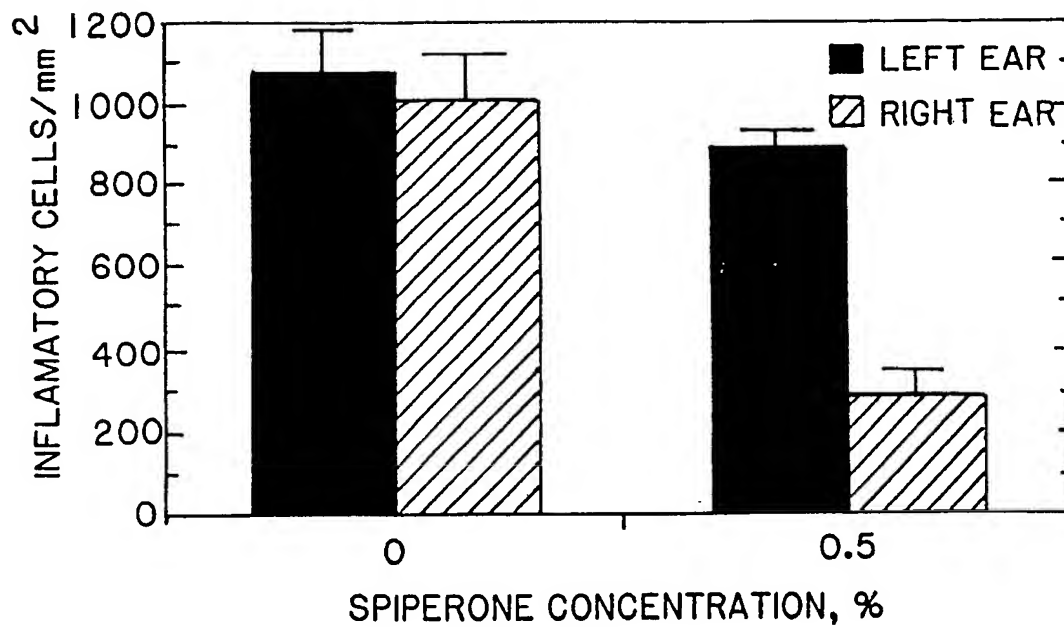
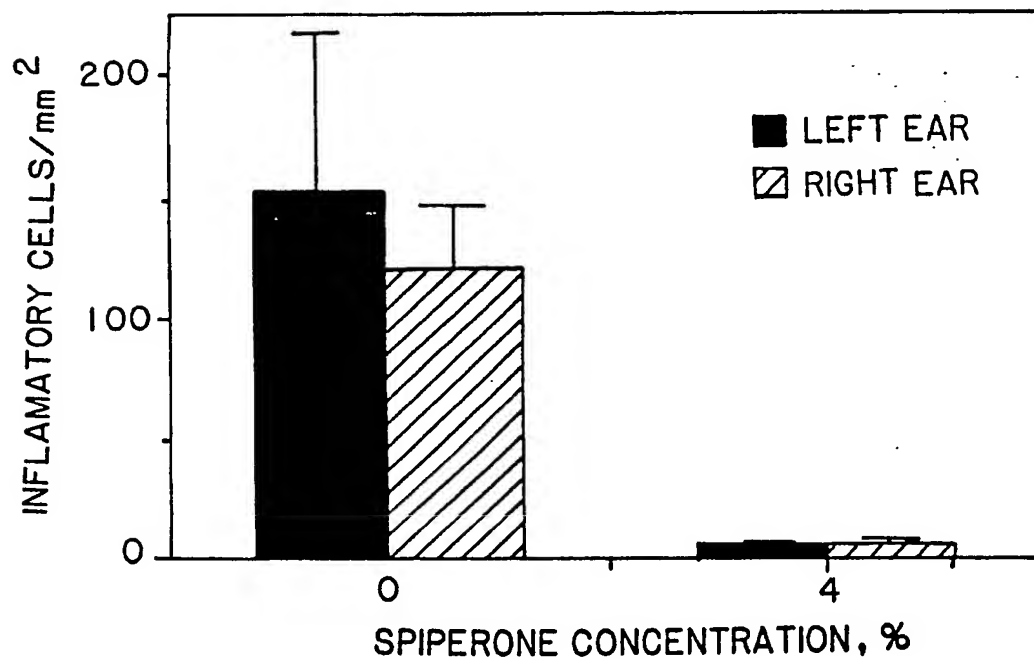
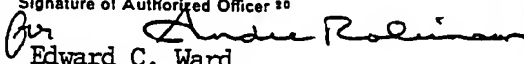


FIG. 6



INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/04637

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³ According to International Patent Classification (IPC) or to both National Classification and IPC IPC (5): A61K 31/44 U.S. CL: 514/280		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁴		
Classification System	Classification Symbols	
U.S.	514/280	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁵		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴		
Category ⁶	Citation of Document, ¹⁵ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
Y	Elozovski et al. Arch intern. Pharmacodynamie 123 58-66 1959, Chemical Abstracts Vol. 54, 1960 Abstract 215049-i.	1 to 6, 10&15 18 and 19
Y	Loffman et al. America Heart Journal, 74(2) 229-34, 1967 Chemical Abstracts vol. 67, 1967, Abstract 81019W	1 to 6, 10-15 18 and 19
X	US,A 2,788,309 Cooper 09 April 1957 See entire document	10 to 15
X	US,A 2,854,380 Jensen et al. 30 September 1958 See entire document	10 to 15
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>[*] Special categories of cited documents: ¹⁵</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search ²		Date of Mailing of this International Search Report ³
26 NOVEMBER 1990		31 DEC 1990
International Searching Authority ¹		Signature of Authorized Officer ²⁰
ISA/US		 Edward C. Ward

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out¹, specifically:

3. ☐ Claim numbers because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING²

This International Searching Authority found multiple inventions in this international application as follows:

Group I, Claims 1 to 6, 10 to 15, 18 and 19 compositions containing reserpine.

Group II, Claims 1 to 4, 10 to 14, 16 to 19 compositions containing spiperone.

Group III, Claims 1 to 19, compositions containing cinanserin

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

1 to 6, 10 to 15, 18 and 19 compositions containing reserpine

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.